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Relationship between Sex Hormone-Binding Globulin and Pregnancy Outcome in Women Undergoing Controlled Ovarian Hyperstimulation for Assisted Reproduction

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Abstract. The purpose of this study was to determine whether the changes of sex hormone-binding globulin (SHBG) affect the pregnancy outcome in women undergoing controlled ovarian hyperstimulation (COH) for assisted reproduction. Forty-five infertile women who were undergoing pituitary desensitization and COH for *in vitro* fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI) and 19 women with normal menstrual cycles participated in the study. Fasting blood samples of the follicular and luteal phases, including follicular fluid during oocyte retrieval, were obtained for determination of estradiol (E_2), progesterone (P_4), testosterone (T), and SHBG concentrations. The SHBG levels increased progressively during the course of COH, but remained constant throughout normal menstrual cycles. A positive correlation existed between E_2 and SHBG levels in both the follicular and luteal phases. The mean plasma SHBG concentration and E_2/T ratio were significantly higher, while the level of T and the free androgen index were significantly lower, in the luteal phase of women who conceived than in those who did not conceive following COH. The changes of follicular fluid SHBG level and E_2/T ratio were similar to those in plasma. We concluded, therefore, that increases in SHBG in the follicular and luteal phases may be a reflection of the functional state of ovarian stimulation, and further that such elevations may influence the pregnancy outcome through the modulation of circulating estrogen and androgen balance during down-regulated COH cycles for IVF/ICSI.

Key words: Sex hormone-binding globulin, Estradiol, Testosterone, *In vitro* fertilization, Intracytoplasmic sperm injection
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SEX hormone-binding globulin (SHBG), primarily produced by the liver, is the major determinant of the biologically available estradiol (E_2) and testosterone (T) in human plasma. SHBG levels are regulated by estrogens, androgens, thyroid hormone, obesity, stress, and genetic factors [1–3]. The plasma concentration of SHBG reflects the balance between estrogen and androgen. No changes in SHBG have been reported to occur during a normal menstrual cycle despite significant increases in preovulatory E_2 levels [1, 4, 5], however, conflicting findings have been reported [6]. A pronounced increase in SHBG levels has been documented in nonpregnant women when circulating E_2 is increased to particularly high levels, as during gonadotropin treatment [4, 5, 7]. Follicular fluid also has high levels of SHBG during ovulation induction, presumably to regulate the bioactivity of ovarian steroids for follicular and embryo development and maturation [8–11]. Furthermore, it has been suggested that SHBG may modulate the bioavailability of ovarian steroids to affect pregnancy outcome during *in vitro* fertilization (IVF) treatment, because follicular fluid SHBG levels are significantly higher in women achieving pregnancy than in those who did not. A decreased bioavailability of E_2 thus has been considered to favor the establishment of pregnancy [12–14]. Taken together, these results imply that SHBG may play a significant role in the pregnancy outcome following controlled ovarian hyperstimulation (COH) for IVF with or with-

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out intracytoplasmic injection (ICSI).

However, whether the supraphysiological E_2 levels which exist during the follicular phase of a down-regulated COH cycle and the exogenous progesterone administration for luteal support during IVF/ICSI are associated with the changes in SHBG concentrations remains unclear. In the current study, the plasma SHBG levels in women with tubal and/or male factors were compared to healthy women with normal menstrual cycles. The influence of SHBG changes on the microenvironment of follicular fluid, the hormonal profile during implantation, and the outcome of pregnancy were evaluated by comparing plasma and follicular fluid ovarian steroid levels between the women who conceived and those who did not conceive after treatment.

Materials and Methods

Study subjects and procedures

Nineteen healthy women aged 24–35 years with a body mass index (BMI) between 19 and 24 kg/m^2 were enrolled in the study. These women had normal menstrual cycles, 28 ± 3 days in length during the preceding 3 months, and thus served as the control group (normal menstrual group). All normal menstrual group subjects had normal basal levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin (PRL) in the early follicular phase. The study was approved by the hospital's review board and informed consent was obtained from each subject. A total of 45 women with tubal and/or male factor infertility who were treated with COH for IVF/ICSI comprised the comparison group (COH group). None of the study participants were taking any medications and none had any systemic diseases or symptoms related to polycystic ovarian disease. There was no history of weight loss or gain exceeding 10% of the registration body weight within six months. All COH group subjects underwent a standard long protocol IVF treatment beginning with intranasal gonadotropin-releasing hormone (GnRH) agonist spray during the mid-luteal phase of the preceding cycle using buserelin 900 μg/day (Suprecur, Hoechst, Frankfurt, Germany) for 2–3 weeks. Purified FSH 225 IU (Serono, Singapore Pte Ltd, Taiwan Branch) was administered daily from the third day of menstruation to the sixth day, and human menopausal gonadotropins (HMG, Serono, Singapore Pte Ltd, Taiwan Branch) were administered daily, from the seventh day until the day of hCG (Profasi, Serono, Singapore Pte Ltd, Taiwan Branch) injection, when the presence of a dominant follicle with a diameter >18 mm was detected. Following oocyte retrieval, conventional IVF was performed for patients with tubal factor, with the addition of ICSI in those receiving treatment for male factor infertility. Early morning (08:00–10:00 AM) fasting blood samples from each subject in the NM and COH groups were collected into chilled tubes containing EDTA and labeled as follows: cycle days (D) 3–5 (early follicular phase), mid-follicular (D7–9), late follicular (D10–11), pre-ovulatory interval (D12–14; day of hCG injection), early luteal (D17–19; day of embryo transfer), mid-luteal (D20–23), and late luteal (D26–28) phases. The pre-ovulatory day of the normal menstrual cycle was determined by urinary LH surge (OvuSign LH, Princeton BioMeditech Corp, Princeton NJ, USA) and then confirmed by an elevation of the plasma LH level after examination by vaginal ultrasonography (Toshiba SSA-340 ACS, Tokyo, Japan) revealed a mature follicle >18 mm in diameter. On the day of oocyte retrieval, blood was collected and follicular fluid was obtained from two representative preovulatory follicles in both ovaries. The fluid was then pooled and centrifuged for 10 minutes at 920 × g, and the supernatant was stored at –20°C for later analysis. Embryo transfer was performed on the third day after oocyte retrieval. Luteal support was administered orally with micronized progesterone 600 mg daily (Utrogestan, Piette, Belgium) and intramuscular progesterone 25 mg/day for approximately 14 days, at which time a pregnancy test was performed.

Hormonal assays

Blood samples were centrifuged immediately after collection, and equal aliquots of plasma were pooled and stored at –20°C until assayed. Plasma E_2, P_4, T, and SHBG levels were determined by RIA (Diagnostic Products Corp, Los Angeles, CA, USA). The inter- and intra-assay coefficients of variation for E_2, P_4, T, and SHBG were 9.3% and 5.7%, 8.5% and 6.2%, 8.7% and 6.8%, and 9.5% and 5.8%, respectively. Samples were assayed in duplicate. The free androgen index (FAI) was calculated using the following formula: [T (nmol/L) × 100]/SHBG (nmol/L).

Statistical analysis

Statistical significance was determined by Student's paired or unpaired *t*-test. Correlations were performed by Pearson's method. A *P* value of less than 0.05 was considered statistically significant. Results are expressed as the mean ± standard error of the mean (SEM).

Results

The patient profiles and mean (±SEM) plasma E_2, P_4, T, and SHBG levels throughout the normal menstrual and COH cycles are summarized in Table 1. Whereas SHBG levels remained constant throughout the normal menstrual cycle, SHBG levels changed during the COH cycle as follows: beginning with a nadir in the early follicular phase, there was a significant increase from the mid-follicular to the late follicular phase, a level which was then sustained until preovulation, followed by a continuous increase in SHBG level to the early luteal phase, peaking in the mid-luteal phase, and finally undergoing a slight decrease in the late luteal phase. There were 15 women who became pregnant after IVF/ICSI treatment. The clinical characteristics and embryo quality on the day of oocyte retrieval of the subjects who conceived and those who did not conceive after treatment are presented in Table 2. No significant differences in the quantity of gonadotropins used, number of oocytes retrieved, mature oocytes, fertilized oocytes, or embryos cleaved were noted between the two groups. As shown in Table 3, the plasma and follicular fluid SHBG level and E_2/T ratio were significantly higher and the FAI was significantly lower in women who conceived as compared with those who did not conceive. A slight, but insignificant, reduction in T level on the day of oocyte retrieval was found in the follicular fluid of subjects with confirmed pregnancies compared to subjects negative for pregnancy.

There was a significant correlation between E_2 and SHBG in the follicular ($r = 0.682$, $P<0.001$, $n = 180$) and luteal phases ($r = 0.536$, $P<0.001$, $n = 135$) of COH cycles, but not in normal menstrual cycles. Significant negative correlations existed between the plasma SHBG and T ($r = 0.437$, $P<0.001$, $n = 180$) and SHBG and FAI ($r = 0.513$, $P<0.001$, $n = 180$) during the follicular phase of COH, and these correlations also existed in the follicular fluid samples. No correlation was demonstrated between P_4 and SHBG in either the normal menstrual or COH cycles. There was no change in BMI in any subject during the period of the study.

As presented in Table 4, the mean SHBG levels and E_2/T ratios were significantly higher and the T levels and FAI values were significantly lower in the luteal

Table 1. Clinical profiles and plasma endocrine data in various phases of normal menstrual and COH cycles in study subjects

Subjects	Age	BMI (kg/m^2)	E_2 (pg/mL)	T (ng/mL)	P_4 (ng/mL)	SHBG (nmol/L)	FAI	E_2/T ratio
Control group (phase) n = 19								
Early follicular	29.5 ± 1.5	21.8 ± 0.8	51.5 ± 8.9	0.43 ± 0.05	0.58 ± 0.04	53.3 ± 3.2	2.8 ± 0.3	0.12 ± 0.01
Midfollicular			93.6 ± 18.3	0.46 ± 0.04	0.59 ± 0.04	55.5 ± 2.9	2.9 ± 0.3	0.20 ± 0.02
Late follicular			143.7 ± 20.3	0.44 ± 0.04	0.53 ± 0.04	54.7 ± 2.5	2.8 ± 0.3	0.33 ± 0.03
Preovulatory			198.5 ± 22.3**	0.52 ± 0.05	0.83 ± 0.04	52.9 ± 2.8	3.4 ± 0.5	0.38 ± 0.05*
Early luteal			95.6 ± 21.5*	0.46 ± 0.04	7.8 ± 0.5**	53.4 ± 3.1	2.9 ± 0.3	0.21 ± 0.02
Midluteal			152.6 ± 16.8**	0.47 ± 0.03	25.6 ± 0.5***	52.8 ± 3.0	3.1 ± 0.3	0.33±0.04*
Late luteal			65.6 ± 10.3*	0.48 ± 0.05	7.5 ± 3.8**	53.6 ± 2.8	3.1 ± 0.4	0.14 ± 0.02
COH group (phase) n = 45								
Early follicular	30.2 ± 1.6	20.9 ± 0.7	49.6 ± 9.3	0.46 ± 0.05	0.62 ± 0.04	53.7 ± 2.8	3.0 ± 0.3	0.11 ± 0.01
Midfollicular			521.3 ± 23.6	0.62 ± 0.06*	0.85 ± 0.05	64.7 ± 4.7*	3.4 ± 0.5	0.80 ± 0.06
Late follicular			1646.5 ± 83.4	0.93 ± 0.05**	0.89 ± 0.05	74.6 ± 5.9**	3.9 ± 0.6	1.77 ± 0.08**
Preovulatory			2485.7 ± 316.5***	1.36 ± 0.07***	1.5 ± 0.07*	88.9 ± 6.2**	5.4 ± 0.7*	1.83 ± 0.06**
Early luteal			879.5 ± 40.6***	0.85 ± 0.06**	78.4 ± 8.8***	89.9 ± 5.9**	3.3 ± 0.5	1.04 ± 0.06**
Midluteal			1563.6 ± 96.7**	0.87 ± 0.05**	98.3 ± 10.7***	95.4 ± 6.9**	3.2 ± 0.5	1.80 ± 0.07**
Late luteal			958.6 ± 39.8**	0.75 ± 0.05**	85.9 ± 9.5***	85.7 ± 5.2**	3.1 ± 0.3	1.28 ± 0.07**

*P<0.05; **P<0.001, ***P<0.0001 vs early follicular phase in each group
All values are expressed as mean ± SEM.

Table 2. Clinical characteristics on the day of oocyte retrieval in women with pregnancy and non-pregnancy following IVF/ICSI

	Pregnancy	Non-pregnancy	*P* value
No. of patients	15	30	
Duration of infertility (years)	4.5 ± 0.4	4.3 ± 0.5	NS
Age (years)	30.9 ± 0.6	30.3 ± 0.5	NS
BMI (kg/m^2)	21.8 ± 0.8	20.2 ± 0.6	NS
FSH on cycle days 3 (mIU/mL)	6.5 ± 0.3	6.4 ± 0.4	NS
No. of ampules used	36.7 ± 0.9	37.3 ± 0.8	NS
No. of oocytes retrieved	11.6 ± 1.0	12.5 ± 0.3	NS
No. of metaphase II oocytes	9.6 ± 0.8	9.7 ± 0.7	NS
No. of fertilized oocytes	6.8 ± 0.5	7.2 ± 0.4	NS
No. of cleaved embryos	6.2 ± 0.6	6.4 ± 0.5	NS
No. of embryos transferred	3.4 ± 0.1	3.5 ± 0.2	NS

All values are expressed as mean ± SEM. NS = not significant.

Table 3. Hormonal data of plasma and follicular fluid on the day of oocyte retrieval in women with pregnancy and non-pregnancy following IVF/ICSI

	Pregnancy	Non-pregnancy	*P* value
Plasma E_2 (pg/mL)	1873.5 ± 289.9	1618.4 ± 255.6	NS
Follicular fluid E_2 (ng/mL)	563.2 ± 38.9	589.5 ± 48.6	NS
Plasma P_4 (ng/mL)	6.8 ± 1.6	6.2 ± 1.5	NS
Follicular fluid P_4 (ng/mL)	6932.5 ± 516.5	6521.2 ± 489.3	NS
Plasma T (ng/mL)	1.4 ± 0.2	1.6 ± 0.3	NS
Follicular fluid T (ng/mL)	5.9 ± 0.5	6.8 ± 0.6	NS
Plasma FAI	5.5 ± 0.7	7.3 ± 1.2	NS
Follicular fluid FAI	31.8 ± 4.5	45.9 ± 6.7	$P<0.05$
Plasma E_2/T ratio	1.34 ± 0.2	1.01 ± 0.2	NS
Follicular E_2/T ratio	95.4 ± 4.5	86.7 ± 4.1	$P<0.05$
Plasma SHBG (nmol/L)	89.8 ± 6.2	76.6 ± 5.6	$P<0.05$
Follicular fluid SHBG (nmol/L)	64.9 ± 3.9	51.8 ± 3.6	$P<0.05$

All values are expressed as mean ± SEM. NS = not significant.

Table 4. Plasma hormonal data in various phases of pregnancy and non-pregnancy following IVF/ICSI

Subjects	E_2 (pg/mL)	T (ng/mL)	P_4 (ng/mL)	SHBG (nmol/L)	FAI	E_2/T ratio
Pregnant group (phase)						
Early follicular	48.9 ± 8.9	0.44 ± 0.05	0.59 ± 0.04	54.3 ± 3.2	2.8 ± 0.3	0.11 ± 0.01
Midfollicular	563.6 ± 18.3	0.66 ± 0.04	0.89 ± 0.05	65.7 ± 2.9	3.5 ± 0.4	0.81 ± 0.04
Late follicular	1683.7 ± 20.3	0.88 ± 0.04	0.89 ± 0.04	76.5 ± 2.5	3.6 ± 0.4	1.91 ± 0.05
Preovulatory	2598.5 ± 322.3	1.31 ± 0.05	1.5 ± 0.07	92.8 ± 7.8	4.9 ± 0.4	1.98 ± 0.08
Early luteal	935.6 ± 21.5	0.76 ± 0.04*	82.8 ± 7.5	97.8 ± 7.1*	2.8 ± 0.3*	1.23 ± 0.05*
Midluteal	1652.6 ± 16.8	0.75 ± 0.03*	105.6 ± 9.5	104.6 ± 8.0*	2.6 ± 0.2*	2.21 ± 0.07*
Late luteal	995.6 ± 10.3	0.67 ± 0.05*	89.5 ± 7.8	98.7 ± 7.8*	2.7 ± 0.2*	1.49 ± 0.05*
Non-pregnant group (phase)						
Early follicular	49.6 ± 9.3	0.47 ± 0.05	0.63 ± 0.04	52.3 ± 2.8	3.1 ± 0.3	0.11 ± 0.01
Midfollicular	482.3 ± 25.6	0.63 ± 0.06	0.85 ± 0.05	63.6 ± 4.7	3.5 ± 0.3	0.79 ± 0.05
Late follicular	1548.5 ± 73.4	0.98 ± 0.05	0.88 ± 0.05	72.6 ± 5.9	4.2 ± 0.4	1.58 ± 0.07
Preovulatory	2375.7 ± 284.5	1.41 ± 0.06	1.5 ± 0.06	84.7 ± 6.2	5.8 ± 0.5	1.68 ± 0.06
Early luteal	823.5 ± 39.6	0.95 ± 0.06	74.3 ± 6.8	80.8 ± 5.9	4.0 ± 0.3	0.87 ± 0.04
Midluteal	1473.5 ± 86.5	0.99 ± 0.05	92.3 ± 8.7	86.3 ± 6.9	3.9 ± 0.3	1.48 ± 0.07
Late luteal	921.5 ± 40.7	0.84 ± 0.05	81.9 ± 6.1	82.5 ± 5.2	3.4 ± 0.3	1.11 ± 0.05

*P<0.01, significant difference as compared with the corresponding value in non-pregnant group.
All values are expressed as mean ± SEM.

phase of those with a successful pregnancy as compared to those who did not conceive after IVF/ICSI treatment.

Discussion

The present study demonstrates that increases in plasma SHBG levels occur throughout the follicular and luteal phases of COH cycles for assisted reproduction. Because on the day of embryo transfer a higher SHBG level and E_2/T ratio and a lower T level and FAI value characterized the pregnant subjects as compared with the nonpregnant subjects, it is possible that SHBG influences the relative circulating estrogen and androgen balance, resulting in superior uterine receptivity for embryo implantation during the luteal phase. Development of uterine receptivity of the endometrium during implantation is affected by the ovarian steroids, estrogen and progesterone [15], and other biomarkers

[16]. The endometrium undergoes marked developmental changes during the proliferative to secretory phases of the cycle. E_2 has been shown to be a mitogenic hormone that leads to growth of the endometrial glands and stroma, and induction of receptors for E_2 and P_4, as required for the maturation process [17, 18]. SHBG is the major circulating high affinity sex steroid binding protein in human plasma and the endometrium [15]. SHBG functions to balance the levels of testosterone, E_2, and P_4 during the normal menstrual cycle [1–3]. Therefore, it seems likely that an increase of SHBG binding capacity during COH plays a crucial role in improving uterine receptivity of the endometrium for embryo implantation through the modulation of circulating E_2, T, and P_4 bioavailability.

In this study, a relatively low estrogen to androgen ratio was demonstrated in the follicular fluid and during the luteal phase of unsuccessful IVF/ICSI attempts, even though there were no significant differences between the number of mature oocytes, oocytes fertilized, and embryos cleaved when compared to successful attempts. It is unclear to the relationship of subsequent embryo development, if any. However, there is good evidence in animal models for a direct effect of androgens on follicular quality and oocyte maturation, in that exogenous administration of androgen results in follicular atresia, degenerated oocytes, and poor embryo development [11, 19]. Furthermore, it has been recently reported that ovarian stimulation with buserelin and hMG induced an imbalance in endometrial estrogen and progesterone receptors which led to premature secretory endometrial transformation [20]. In addition, the synthesis and secretion of SHBG from the liver are stimulated by hyperproduced E_2 in COH cycles. The SHBG binds to T and inactivate androgen action, which can be beneficial for oocyte quality. Thus the women whose response of SHBG synthesis and secretion to estrogens are high, will get more chance to prepare better oocytes for implantation. It is therefore reasonable to assume that estrogen and androgen imbalance might subsequently result in decreased implantation capacity during the luteal phase and virtually interrupting implantation of the embryo in the endometrium. Based on these collective findings, we propose that SHBG and ovarian steroid levels may be valid markers of pregnancy outcome during IVF/ICSI treatment. However, the influence of SHBG and androgen excess on the oocyte quality such as patients with polycystic ovary syndrome should be examined in the next work.

The results of our study indicate that the E_2 fluctuations which occur during the normal menstrual cycle do not affect SHBG levels. Our results are consistent with those of previous investigators [1, 4, 5] but differ from those who reported SHBG levels were increased during the preovulatory and luteal phases of the normal menstrual cycle [6]. The discrepancy regarding SHBG production appears to reflect the individual's metabolic and nutritional status, growth, and aging rather than temporary fluctuations in sex steroids [21], and/or is explained by genetic factors [22, 23].

Because of the close relationship between changes in plasma E_2 and SHBG levels in the follicular and luteal phases as observed in the present study, it seems most likely that the increase in SHBG binding capacity is due to the influence of an increasing secretion of supraphysiological E_2 on hepatic synthesis. In addition, although supraphysiological E_2 levels were lower in the luteal phase than in the follicular phase in our study, the increase of SHBG was more pronounced during the luteal phase. This finding indicated that E_2 might collaborate with exogenous and endogenous P_4 to stimulate the increase in SHBG during IVF/ICSI treatment. It also suggests that high levels of E_2 may have a priming effect on the liver to sensitize these cells during the follicular phase for the subsequent stimulation of P_4 to effect greater production of SHBG during the luteal phase. Our present findings are consistent with those of previous investigations [4, 5] which have demonstrated that the increase in SHBG level was caused by particularly higher levels of endogenous E_2 during gonadotropin-stimulated menstrual cycles, but GnRH agonists were not used for pituitary desensitization nor was the role of exogenous P_4 on SHBG changes and pregnancy outcome described.

In conclusion, supraphysiological levels of E_2 might cause the increase of SHBG levels during the follicular phase and exogenous P_4 administration for luteal support may have had a synergistic effect with E_2 on the stimulation of SHBG production during the luteal phase of COH cycles. The increase of SHBG in plasma and follicular fluid might affect the pregnancy outcome through the modulation of circulating estrogen and androgen balance during COH cycles for IVF/ICSI. It is suggested that SHBG may serve as a predictor of successful pregnancy in down-regulated women undergoing assisted reproduction.

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